Regulation of receptors, transporters and ion channels by the Nedd4 family of ubiquitin ligases

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Protein modification by ubiquination controls a number of cellular signalling processes, including protein trafficking and turnover. The process of ubiquitination requires a number of enzymes that include a ubiquitin activating enzyme (E1), ubiquitin conjugating enzymes (E2s) and ubiquitin protein ligases (E3s) (reviewed in Ciechanover, 2005). The specificity of the ubiquitination system is determined by E3s, as they are involved in the transfer of ubiquitin from an E2 to a specific substrate. There are two main types of E3s: the RING type, that act as a large complex containing several proteins, and the HECT type, that act as single chain enzymes that first accept the ubiquitin and then transfer it to the substrate protein. Among HECT ligases, the members of Nedd4 family are characterized by a unique modular domain structure containing a C2 domain, 2-4 WW domains and a C-terminal HECT domain (reviewed in Shearwin-Whyatt *et al.*, 2006). Studies in yeast and mammals suggest that many members of this family are involved in the control of trafficking and endocytosis of membrane associated proteins, including ion channels, transporters and receptors (Ciechanover, 2005). We are studying several members of the Nedd4 family and their physiological functions using biochemical, cellular, physiological and gene knockout (KO) approaches. Our recent data suggest that KO of Nedd4 results in growth retardation and perinatal lethality in mice (Cao *et al.*, 2008). The growth phenotype is due to reduced IGF-I signalling caused by reduced levels of IGF-1R on cell surface. This appears to be caused by increased levels of the adaptor protein Grb10, a known inhibitor of IGF-I signalling (Cao *et al.*, 2008). Thus, Nedd4 is an E3 that controls animal growth. Knockout of Nedd4-2 also resulted in perinatal lethality in most animals, but a few homozygous animals survive for up to 3 weeks. Unlike Nedd4 KOs, Nedd4-2 deficient animals seem to develop normally, but die due to a collapsed lung phenotype. The survivors develop lung infections and die due to severe inflammation of the lungs. These phenotypes are consistent with increased activity of the epithelial sodium channel (ENaC) in the Nedd4-2 KO mice. ENaC has been shown previously to be regulated by Nedd4-2-mediated ubiquitination *in vitro* (Harvey *et al.*, 2001; Kamynina *et al.*, 2001; Fotia *et al.*, 2003). In support of this prediction, we found increased cell surface expression of ENaC in the lung and kidney epithelia. Further studies to delineate the *in vivo* function of Nedd4-2 in regulating ENaC and other channels are currently underway.

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